

Supplemental Figure legends

Supplemental Figure 1. Basement membrane [Periodic acid-Schiff (PAS) stained] in ovarian tissue in CTL (A) and DHT-treated rats (B). Basement membrane (BM) was present between granulosa and theca cell layers in follicles at all different stages of development in CTL rats. However, DHT-treated rats exhibited distinct reduction in the antral volume or absence of the antrum, decreased granulosa cell numbers and retention of the theca layer and basement membrane localized in the inner core of the atypical follicles. Arrows indicate location of BM between granulosa and theca layers. CTL, control; DHT, dihydrotestosterone; BM, basement membrane; PAF, preantral follicle; AF, antral follicle; AtyF, atypical follicle; CL, corpus luteum; GC, granulosa cells; TC, theca cells.

Supplemental Figure 2. Fluorescent immunolocalization of NR5A1 in CTL and DHT-treated rats (200X magnification). Ovarian tissue sections were immunostained for NR5A1 in CTL groups (A) and DHT-treated rats (B). Panels A (f-j) and B (d-f) show merged images with nuclear stain (DAPI). The expression of NR5A1 in CTL groups was detected in the nuclei of theca cells but not granulosa cells in preantral, antral and atretic follicles. In contrast, NR5A1 is expressed in both granulosa and theca cells in preovulatory follicles. DHT-treated rats revealed that NR5A1 is expressed in theca cells of antral and atypical follicles but not granulosa cells. CTL, control; DHT, dihydrotestosterone; F, follicle; GC, granulosa cells; TC, theca cells.

Supplemental Figure 3. Granulosa cell aromatase expression is down-regulated with a concurrently increase in apoptosis in the follicles of DHT-treated rats. Double-staining with aromatase (a and e) and active caspase-3 (b and f) in antral follicles in CTL and DHT-treated rats. Panels (c and g) show merged images with DAPI staining. H&E staining on adjacent sections of follicles in CTL (d) and DHT-treated rat (h), respectively (200X magnification). Granulosa cell aromatase expression is down-regulated, while active caspase-3 content is increased in granulosa cell of antral follicles in DHT-treated rats. Black arrows indicate the detached granulosa cells from theca layer (h). GC, granulosa cells; TC, theca cells; H&E, hematoxylin and eosin stain.

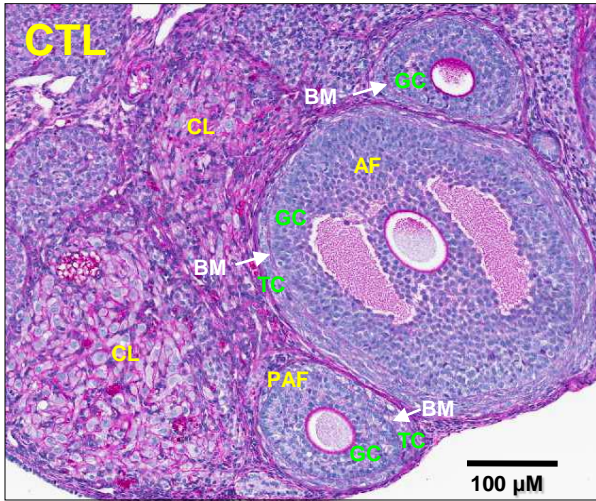
Supplemental Figure 4. DHT induces apoptosis via caspase-3 activation *in vitro*. (A) Granulosa cells were plated on poly-D-lysine coated 8-well glass culture slides and cultured with DHT (10 μ M, 0-96 h). Cells were immunostained (400X magnification) for active caspase-3 (a and d) after DHT treatment (10 μ M, 96 h) and selected areas of merged images (f) with active caspase-3, actin and DAPI (nuclear) are shown at increased magnification (g and h). Actin (cytosolic marker) was detected with red fluorescent Texas Red-X phalloidin (1:100). Yellow arrows indicate active caspase-3 (green, g) and nuclear fragmentation (blue, h), respectively. (B) Cells with active caspase-3 (positive signal, green) were expressed as a percentage of total cells counted. Results are expressed as mean \pm SEM (n = 3 independent experiments). (Data were analyzed by two-way ANOVA followed by Bonferroni *post hoc* test, *** p<0.001, compared to CTL groups) CTL, control; DHT, dihydrotestosterone.

Supplemental Figure 5. Ovarian expression of chemerin, CMKLR1 and GDF9 during follicular development. (A-B) Sections of ovarian follicle at different stages of follicular growth were immunostained for chemerin (A; a-d), CMKLR1 (A; k-n) and GDF9 (B; a-d). Negative controls [blocking peptides for chemerin (A; e), CMKLR1 (A; o) and GDF9 (B; e)] are shown. Panels f-j and p-t show DAPI staining. The chemerin and CMKLR1 are expressed in granulosa and theca cells, and oocytes at all stages of follicular development. GDF9 is expressed only in oocyte, with expression increases with follicular maturation. (C) Expression of GDF9 in CTL and DHT-treated rats *in vivo*. Oocyte GDF9 level is lower in antral follicle in DHT-treated rats than CTL. Ovary tissue sections were immunostained for GDF9 (C; a-b). Blocking peptide for GDF9 as a negative CTL (C; c) is shown. (D) Chemerin suppressed oocyte GDF9 expression in rat follicle *in vitro*. Follicles (diameter, 150–180 μ m) isolated from 14-day old rats were cultured with chemerin (0, 50, 100 and 1000 ng/ml) for four days and follicle sections were

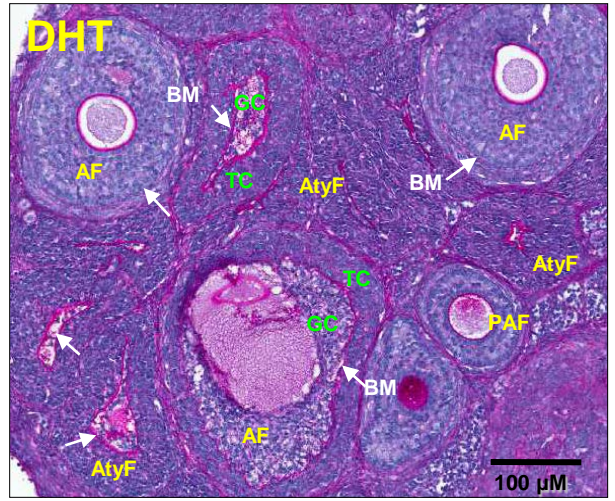
immunostained with GDF9 antibody (negative CTL, specific blocking peptide). CTL, control; DHT, dihydrotestosterone; F, follicle; GC, granulosa cells; TC, theca cells; OO, oocyte; An, antrum.

Supplemental Figure 1

A

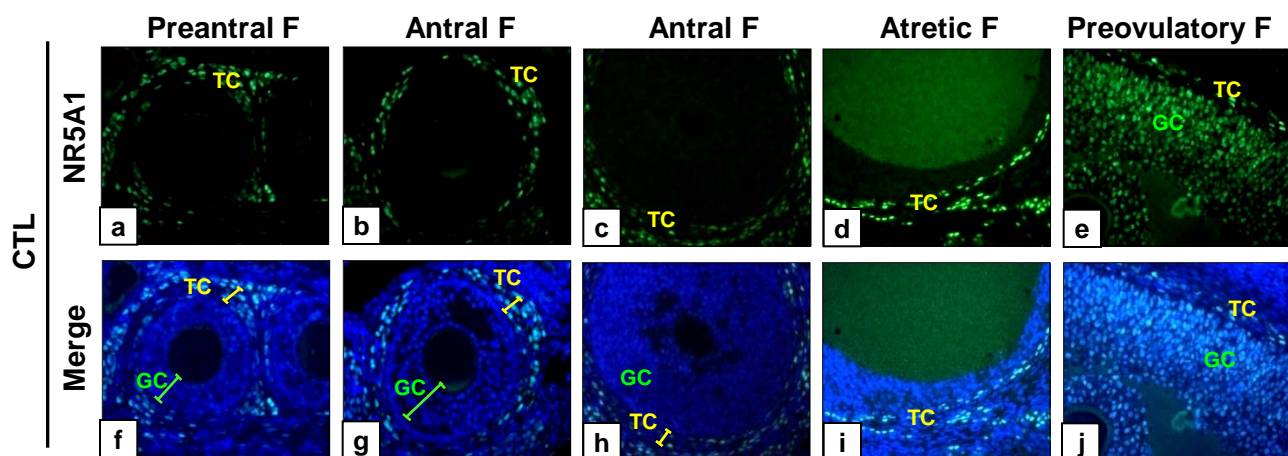


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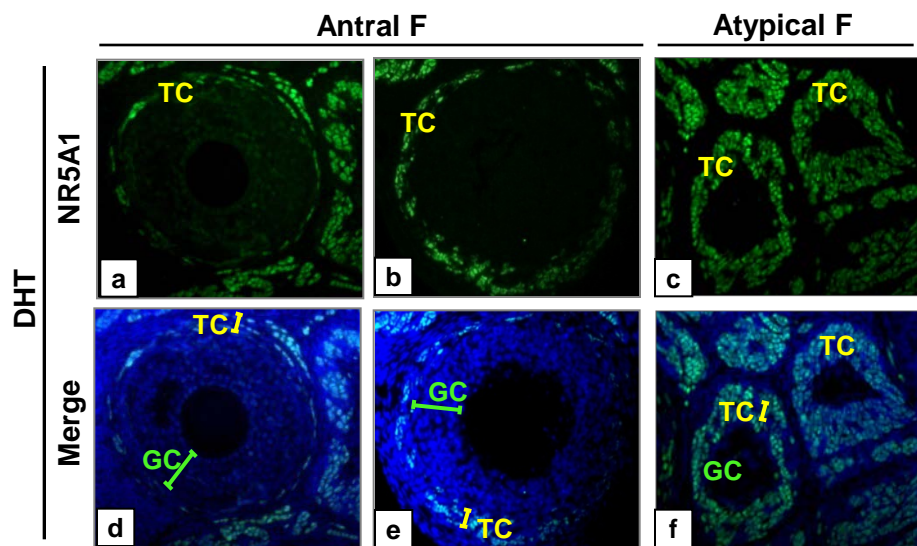


Supplemental Figure 2

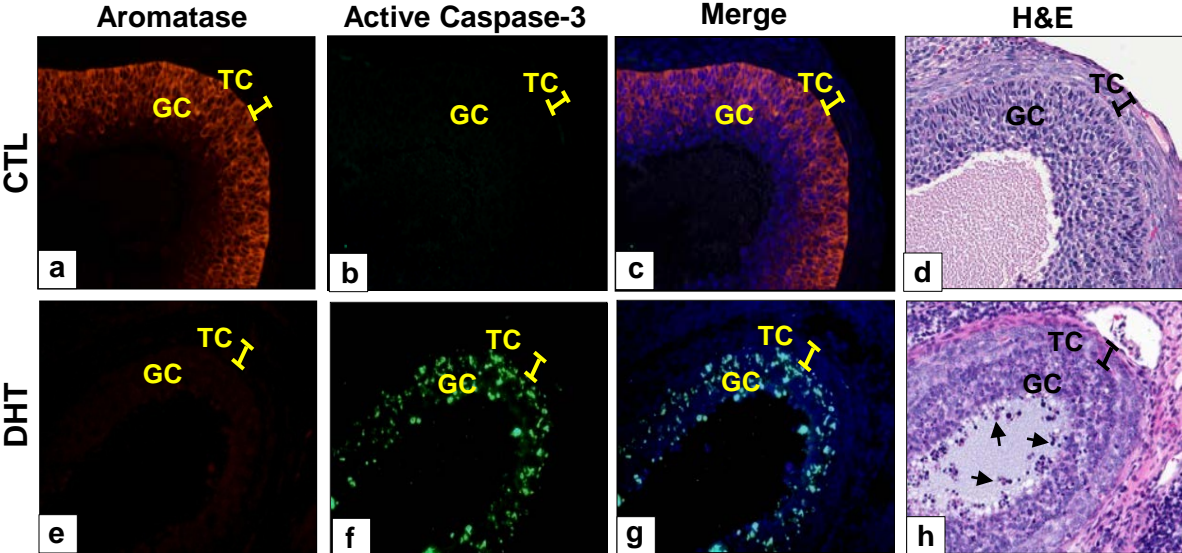
A



B

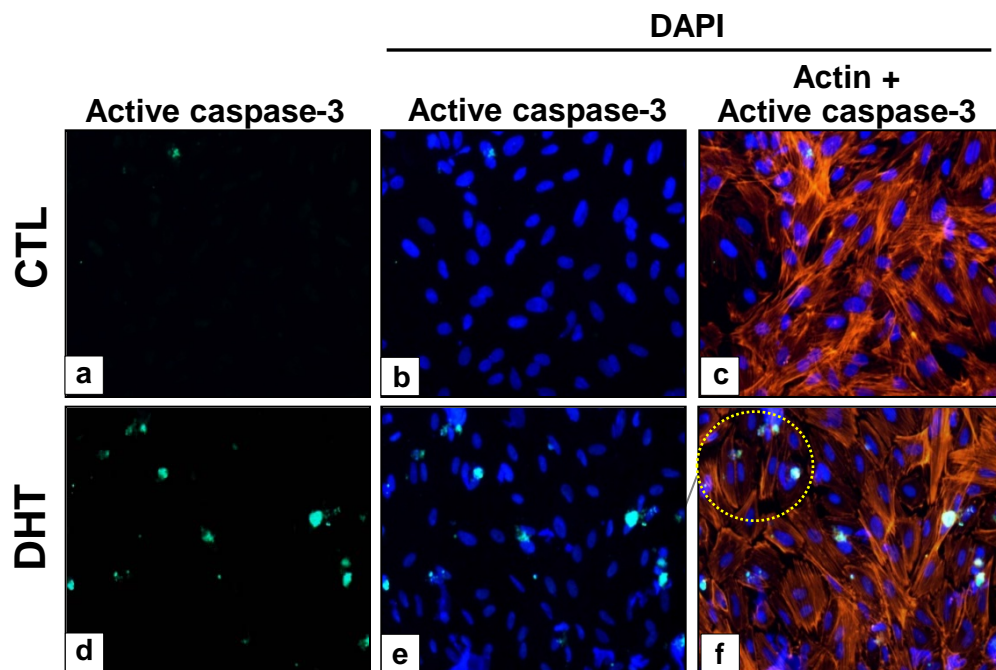


Supplemental Figure 3

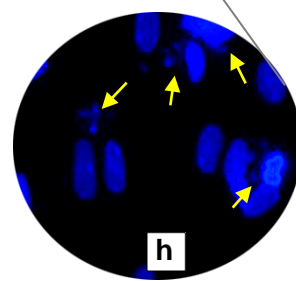
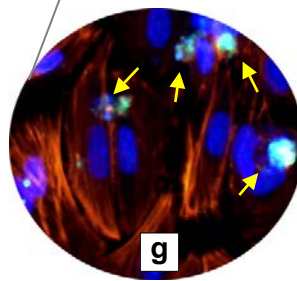
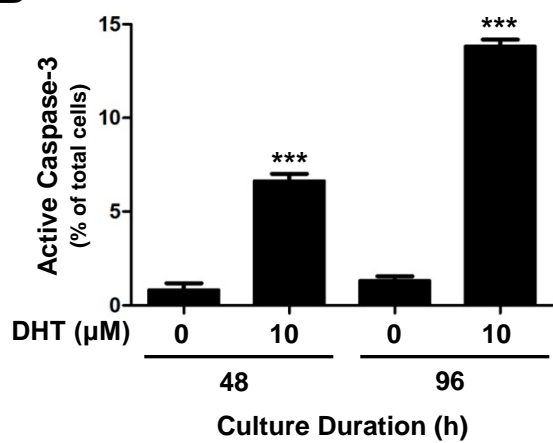


Supplemental Figure 4

A



B



Supplemental Figure 5

